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**APPLICANT: LUTZ HEUER ET AL**
**SERIAL NO.: 09/901,979**
**FILED: JULY 10, 2001**
**FOR: MICROBICIDAL COMPOSITIONS**

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# DECLARATION

I, Martin Kugler, resident at Am Kloster 47, D-42799 Leichlingen, Germany declare:

that I am a biologist having studied at the University of Tübingen;

that I received the degree of doctor rer. nat. at the University of Tübingen in the year of 1986;

that since 1987 I am an employee of Bayer Aktiengesellschaft, Leverkusen, Germany, where I am still working in the department of research of technical materials protecting agents located at Krefeld, Germany;

that I am a senior research microbiologist having 16 years experiences in the field of testing and evaluation of chemical compounds for their action on microbes attacking technical materials;

that I am one of the inventors of the above-identified application;

that the following tests have been carried out under my supervision and direction:

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## Test 1

**Synergism of Cyproconazole / Propiconazole**

Pieces of mycelium were punched out of a colony of the wood-destroying fungus **Gloeophyllum trabeum** and incubated on a nutrient agar containing malt extract/peptone at 26°C. The hyphal growth with and without the addition of the active compound was compared. The minimum inhibitory concentration (MIC) was recorded as the concentration of active compound which completely suppresses radial hyphal growth.

According to the method described by Kull et al. (F.C. Kull, P.C. Eismann, H.D. Sylvestrowicz, R. L. Mayer, Applied Microbiology 9, 538 to 541, 1961) the synergism was then determined. The following equation for the determination of the synergistic index X applies:

$$\frac{Q_A}{Q_a} + \frac{Q_B}{Q_b} = X$$

X = 1 = additivity

X > 1 = antagonism

X < 1 = synergism

$Q_a$ = the MIC of substance A

$Q_b$ = the MIC of substance B

$Q_A$ = the concentration of substance A in the concentration of A/B which suppresses microbial growth

$Q_B$ = the concentration of substance B in the concentration of A/B which suppresses microbial growth

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## Results:

|   |   | **MIC** |
|---|---|---|
| A | cyproconazole | 0.3 ppm |
| B | propiconazole | 3.0 ppm |
| A/B | cyproconazole/propiconazole (2:1) | 0.3 ppm |
| A/B | cyproconazole/propiconazole (1:1) | 0.5 ppm |
| A/B | cyproconazole/propiconazole (1:2) | 0.5 ppm |

By using the above values for cyproconazole/propiconazole

**a) for the mixing ratio of 2:1 the synergistic index X was determined:**

$$X = \frac{0.2}{0.3} + \frac{0.1}{3.0} = 0.7$$

**b) for the mixing ratio of 1:1 the synergistic index X was determined:**

$$X = \frac{0.25}{0.3} + \frac{0.25}{3.0} = 0.9$$

**c) for the mixing ratio of 1:2 the synergistic index X was determined:**

$$X = \frac{0.17}{0.3} + \frac{0.34}{3.0} = 0.7$$

Thus, when cyproconazole and propiconazole are mixed, for example, in a ratio of 2:1, 1:1 and 1:2 a high degree of synergism occurs.

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## Test 2

**Synergism of Cyproconazole / Tebuconazole**

Pieces of mycelium were punched out of a colony of the wood-destroying fungus
**Gloephyllum trabeum** and incubated on a nutrient agar containing malt
extract/peptone at 26°C. The hyphal growth with and without the addition of the
active compound was compared. The minimum inhibitory concentration (MIC) was
recorded as the concentration of active compound which completely suppresses
radial hyphal growth.

The synergistic index X was determined according to the method described in Test 1.

## Results:

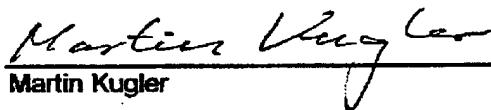|     |                                    | MIC      | X    |
|-----|------------------------------------|----------|------|
| A   | cyproconazole                      | 0.3 ppm  |      |
| B   | tebuconazole                       | 0.5 ppm  |      |
| A/B | cyproconazole/tebuconazole (2:1)   | 0.3 ppm  | 0.86 |
| A/B | cyproconazole/tebuconazole (1:1)   | 0.3 ppm  | 0.8  |
| A/B | cyproconazole/tebuconazole (1:2)   | 0.3 ppm  | 0.73 |

Thus, when cyproconazole and tebuconazole are mixed, for example, in a ratio of
**2:1, 1:1 and 1:2** a high degree of synergism occurs.

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The foregoing results, in my opinion, provide proof that not only the tested ratios, but all ratios of cyproconazole and propiconazole and all ratios of cyproconazole and tebuconazole would be expected to be synergistic. As persons skilled in the art know, synergism depends on the components that are mixed, not on the ratios of such components. Accordingly, by showing that cyproconazole and propiconazole and cyproconazole and tebuconazole show synergism at the demonstrated ratios, we have, in fact, established the expectation that cyproconazole and propiconazole and cyproconazole and tebuconazole will be synergistic at all ratios.

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Leverkusen, Germany, this    day of  2005 - 12 - 15

_Martin Kugler_
Martin Kugler